PIONEER HI-BRED INTERNATIONAL, INC.
CORPORATE INTELLECTUAL PROPERTY DEPARTMENT

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TO: Commissioner for Patents

FROM: Steven Callistein

RE: Transposable Element-Anchored Amplification Method of Isolation and Identification of Tagged Genes
Case 0457 EE

DATE: 7/16/2004 FAX NUMBER: (703) 305-0942

NUMBER OF PAGE(S) FOLLOWING THIS SHEET: 16

COMMENTS:
1. Fee Transmittal Form – 1 page
2. Appeal Brief – 15 pages

PTO/SB/17 (10-03)
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☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 330

Complete if Known	
Application Number	09/622,353
Filing Date	September 12, 2000
First Named Inventor	John A Arbuckle et al.
Examiner Name	Joyce Tung
Art Unit	1638
Attorney Docket No.	0457 EE

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 16-1052

Deposit Account Name: Pioneer Hi-Bred International, Inc.

The Director is authorized to: *(check all that apply)*

☒ Charge fee(s) indicated below ☐ Credit any overpayments

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 0

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims		- ** =	0	X		-	0
Independent Claims		- ** =	0	X		=	0
Multiple Dependent				X		=	0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	330.00
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,330	2453	665	Petition to revive – unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17 (q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) ____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) 330

SUBMITTED BY					Complete (if applicable)
Name (Print/Type)	Steven Callistein	Registration No. (Attorney/Agent)	43,525	Telephone	(515) 254-2823
Signature				Date	July 16, 2004

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

IN RE: Arbuckle, John A., et al.	
SERIAL NO: 09/622,353	APPEAL NO. _____
FOR: Transposable Element-Anchored Amplification Method of Isolation and Identification of Tagged Genes	BRIEF ON APPEAL
FILED: September 12, 2000	
EXAMINER: Joyce Tung	
GROUP ART UNIT: 1638	
ATTORNEY DOCKET: 0457 EE	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

This brief is in furtherance of the Notice of Appeal filed in this case on June 3, 2004.

The fees required under § 1.17, and any required petition for extension of time for filing this brief, and fees therefore, are dealt with in the accompanying Transmittal of Appeal Brief.

This brief is transmitted in triplicate.

Real Party in Interest

The subject application is owned by Pioneer Hi-Bred International, Inc. of Des Moines, Iowa.

Related Appeals and Interferences

To the best of my knowledge there are no related appeals or interferences that will directly affect or be directly affected by, or have a bearing on, the Board of Appeals decision in the pending appeal.

Status of Claims

This is an appeal from the Final Rejection of claims 1-21 dated March 18, 2004.

Claims 1, 4-7, 9-13 and 15-21 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998).

Claims 2 and 8 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998) as applied to claims 1, 4-7, 9-13, and 15-21, and further in view of Schunable et al. [Schnable et al.] (5,684,242, issued 11/1997).

Claims 3 and 14 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Lindemann et al. (5,958,738, issued 9/1999) in view of (Kindiger et al. (5,710,367, issued 1/1998) as applied to claims 1,4-7,9-13, and 15-21, and further in view of Halverson et al. (5,707,809, issued 1/1998).

The claims on appeal are set forth in the attached Appendix. A history of claim amendments is as follows: Claims 1, 6, 9-15 and 17 were amended on 9/11/2001; Claims 9 and 17 were amended on 1/28/2002; Claims 9 and 17 were amended on 4/11/2002; and Claim 2 was amended on 10/4/2002.

Status of Amendments

No amendment was filed subsequent to the Final Rejection of the claims.

Summary of the Invention

Transposable elements present in a genome may disrupt gene function and cause desired phenotypes. When a desired phenotype of interest is identified it is also desirable to identify the gene affected by the transposable element. The present invention provides a novel method for the isolation and identification of such gene by use of transposable element-anchored amplification.

The method of the invention uses the transposable element as an anchor for a first primer site. A second primer site is produced by fragmentation of the genome and the attachment of an adapter sequence to the fragments. Amplification is then carried out using these two primer sites. This method provides for fewer laboratory manipulations than previous methods that use Southern analysis and a subtractive hybridization process and has the further advantage of enriching for the genetic sequences affected by the insertion of the transposable element.

Issues

1. Whether the disclosures of Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998) teach or suggest amplification employing a first oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said transposable element, as required by step (e) of claim 1?

2. Whether the requisite motivation for modifying and combining the disclosures of Lindemann et al. (5,958,738, issued 9/1999) and Kindiger et al. (5,710,367, issued 1/1998) has been sufficiently provided to establish a prima facie case of obviousness under 35 USC §103(a) for the invention in claims 1, 4-7, 9-13 and 15-21?

3. Whether the disclosures of Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998) and in further view of Schnable et al. (5,684,242, issued 11/1997) may be properly combined to establish a prima facie case of obviousness under 35 USC §103(a) for the invention in claims 2 and 8?

4. Whether the disclosures of Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998) and in further view of Halverson et al. (5,707,809, issued 1/1998) may be properly combined to establish a prima facie case of obviousness under 35 USC §103(a) for the invention in claims 3 and 14?

Grouping of the Claims

The claims do not stand or fall together. The patentability of the claims will be argued separately.

Arguments

Introduction

Applicants submit that a proper prima facie case of obviousness has not been established as required by M.P.E.P. §2143. First, the prior art references, when combined, do not teach or suggest all of the elements of the claimed invention. Second, no suggestion or motivation to modify the teachings or combine the reference teachings has been provided.

Issue 1

1. Whether the disclosures of Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998) teach or suggest amplification employing a first oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said transposable element, as required by step (e) of claim 1?

The Examiner has not established a prima facie case of obviousness under 35 USC §103(a) for the invention in claims 1, 4-7, 9-13 and 15-21, because the references do not teach all elements of the claimed invention. Neither Lindemann et al. nor Kindiger et al. teach or suggest amplification employing a first oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second oligonucleotide primer which selectively hybridizes

under stringent hybridization conditions to said transposable element, as required by step (e) of claim 1.

In the office action dated 08/21/2003, the Examiner acknowledges that Lindemann et al. do not disclose an oligonucleotide primer which hybridizes under stringent hybridization conditions to the transposable element in a genetic sequence.

The Examiner then turns to Kindiger et al. to supply this missing claim element. The Examiner, paraphrasing Kindiger et al., states in the office action on page 3 lines 17-22 that "One transposable element termed mutator "Mu" is particularly active and has been used successfully to locate the position of genes as well as providing a marker for their isolation (See column 12, lines 46-50). The A and N gene can be isolated and cloned via the 220 bp terminal inverted repeat "flag" used to identify a Mu insertion. The Mu probe is used to identify the mutant for the gene (See column 13, lines 9-18)." (emphasis added) The Examiner then concludes in the following paragraph that "One of ordinary skill in the art at the time of the instant invention would have been motivated to modify the method of Lindemann et al. by applying the Kindiger et al. ' s primer which hybridizes to the transposable element for the identification and isolation of a genetic sequence which is disrupted by a transposable element flanking the genetic sequence associated with a mutant phenotype. " (emphasis added). However, Kindiger discloses a probe, not a primer. More specifically, Kindiger et al. does not teach using a primer that selectively hybridizes to the transposable element. On column 13 lines 13-15, Kindiger et al. state that "When a mutant for the apomixis gene is found, the first step in locating the gene would be to probe a Southern blot containing DNA from the mutated stock with a publicly available Mu1 probe." (emphasis added). Probing by Southern blot is a prior art method that does not utilize an amplification primer that hybridizes to the transposable element or to an adapter sequence. This is further clarified in Kindiger et al. in column 12 lines 57-60, when Kindiger et al. teach that "Mu leaves a particular genetic fingerprint at that locus; thereby enabling isolation of the corresponding region and gene by established methods (Chandler et al., 1994; Martienssen et al., 1989; and O'Reilly et al., 1985)." (emphasis added). Thus, Kindiger et al. envision only the use of the Mu probe in established methods and do not suggest or teach utilizing primers that hybridize to the transposable element and to an adapter

sequence. Applicants' method is an improvement over the established methods described in Kindiger et al. because, among other things, Applicants' method does not require probing a Southern blot.

Issue 2

2. Whether the requisite motivation for combining and modifying the disclosures of Lindemann et al. (5,958,738, issued 9/1999) and Kindiger et al. (5,710,367, issued 1/1998) has been sufficiently provided to establish a prima facie case of obviousness under 35 USC §103(a) for the invention in claims 1, 4-7, 9-13 and 15-21?

To establish a *prima facie* case of obviousness, there must be some suggestion or motivation, either in the references or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. The patent office has the burden of proof in establishing a *prima facie* case of obviousness. M.P.E.P. §2143.

In asserting a prima facie case of obviousness, the Examiner concludes that "One of ordinary skill in the art at the time of the instant invention would have been motivated to modify the method of Lindemann et al. by applying the Kindiger et al.'s primer ..." (Final Rejection dated 3/18/2004, at line 23).

As discussed in issue 1 above, Kindiger et al. does not provide any motivation to modify Lindemann et al. Kindiger et al. expressly acknowledge that they are using only established methods to identify the Apomixis genes. They then cite to Chandler et al., 1994; Martienssen et al., 1989; and O'Reilly et al., 1985 as further confirmation of what they mean by established methods. None of the three references cited by Kindiger et al. disclose Applicants' invention as claimed, nor do any of these references suggest ways to achieve the advantages of the Applicants' method. The Applicants' method eliminates the time and expense involved in Southern analysis, library construction and subcloning associated with the referenced methods. (Specification, page 2, lines 20-23). Kindiger et al. specifically requires Southern blotting (Kindiger et al. column 13, lines 12-19). Although not expressly stated, the

method discussed in Kindiger et al. also requires library construction and subcloning to identify the sequence.

The Applicants also point out that their invention as claimed is not simply the method of Lindemann et al. plus additional steps. While the Examiner has attempted to provide additional references to supplement Lindemann et al., no suggestion or motivation has been provided as to why one of ordinary skill in the art would also choose to delete steps that are part of the method of Lindemann et al. For example, Lindemann et al. requires a subtractive hybridization step that involves a physical mixing of DNA. This step is not a necessary aspect of Applicants' claimed invention. To establish a *prima facie* case of obviousness there must also be some evidence to suggest why one of skill in the art would be motivated to modify Lindemann et al. in order to arrive at Applicants' claimed invention.

In the interview of 3/12/03, Dr. Meeley described how the transposon-anchored amplification method claimed by Applicants is novel and non-obvious with respect to Lindemann *et al.* First, Applicants' invention recites the use of transposon-anchored PCR primers. These are directed toward specific genetic sequences and are used in combination with primers directed toward an adapter sequence. In contrast, the invention of Lindemann *et al.* relies on primers directed to adapters only, and does not contemplate their use in combination with primers anchored to genetic sequences. Second, Lindemann *et al.* specifies the use of one or more subtractive hybridization steps that are unnecessary and avoided in the presently claimed invention.

Thus, the Applicants do not see how the prior art references provide a basis for either modifying or combining the references to achieve Applicants' claimed invention. The patent office has not met its burden of establishing how or why a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select and modify the elements from the cited prior art references for combination in the manner claimed. The cited prior art references do not acknowledge the desirability of solving the problems that have been solved by Applicants' invention. Further, even assuming *arguendo* that there is a motivation to combine Lindemann et al. and Kindiger et al., these two references

they would still be missing elements of the claimed invention and would also contain elements that have been eliminated in Applicants' claimed invention.

The motivation to combine or modify the references cannot be taken from Applicant's specification. It is impermissible to employ hindsight reconstruction using the Applicants' disclosure as a blueprint to reconstruct the claimed invention from isolated pieces of the prior art. See, *Grain Processing Corp v. American Maize-Prods*, 5 USPQ2d 1788, 1792 (Fed. Cir. 1988) and *In re Dembiczak*, 50 USPQ2d 1614 (Fed. Cir. 1999). Absent the motivation provided by Applicants' disclosure, no motivation to combine or modify the references has been provided and a prima facie case of obviousness has not been established.

Issue 3

3. Whether the disclosures of Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998) and in further view of Schnable et al. (5,684,242, issued 11/1997) may be properly combined to establish a prima facie case of obviousness under 35 USC §103(a) for the invention in claims 2 and 8?

The Examiner rejects claims 2 and 8 in further view of Schnable et al.

Claim 2 is a dependent claim wherein step (f) comprises the use of co-segregation analysis. The Examiner states that "Lindemann et al. do not disclose using cosegregation analysis to isolate DNA amplification product that cosegregates with the mutant phenotype." (Final Rejection dated 3/18/2004, page 4, lines 15-16). The Examiner then cites Schnable et al., column 19, lines 33-43, for the proposition that "cosegregation analysis was performed to isolate the DNA amplified product that cosegregates with the mutant phenotype." (emphasis added). However, the example in Schnable et al. cited by the Examiner involves cosegregation to identify a 3.4 kilobase Mu1 hybridizing restriction fragment present in male sterile siblings but absent in male fertile siblings (column 19, lines 40-43). It does not involve using a primer that selectively hybridizes to the transposable element, nor does it involve the DNA amplified product that would result from the use of such a primer. Rather, Schnable et al. uses Southern blotting, library construction and subcloning to identify

the genetic sequence, and as discussed above, these steps are not necessary to practice Applicants' method.

Claim 8 is a dependent claim to a maize plant. The Examiner cites Schnable et al., column 8, lines 55-58, for the proposition that Schnable et al. uses maize. It is unclear to Applicants how Schnable et al. is being used as additive to Lindemann et al. and Kindiger et al. Nevertheless, the arguments made by the Applicants in connection with the claim rejections under the combination of Lindemann, et al. and Kindiger et al. are equally applicable to the claim rejections under the combination of Lindemann, et al., Kindiger et al. and Schnable et al., and such arguments are hereby incorporated by reference into this section.

Applicants do not see how the prior art references provide a basis for either modifying or combining the references to achieve Applicants' claimed invention. The patent office has not met its burden of establishing how or why a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select and modify the elements from the cited prior art references for combination in the manner claimed. The cited prior art references do not acknowledge the desirability of solving the problems that have been solved by Applicants' invention. Further, even assuming *arguendo* that there is a motivation to combine Lindemann et al., Kindiger et al. and Shnable et al., these three references together still lack elements of the claimed invention and contain elements that have been eliminated in Applicants' invention.

Issue 4

4. Whether the disclosures of Lindemann et al. (5,958,738, issued 9/1999) in view of Kindiger et al. (5,710,367, issued 1/1998) and in further view of Halverson et al. (5,707,809, issued 1/1998) may be properly combined to establish a prima facie case of obviousness under 35 USC §103(a) for the invention in claims 3 and 14?

The Examiner rejects claims 3 and 14 in further view of Halverson et al.

Claim 3 is a dependent claim wherein step (f) comprises the use of bulked segregant analysis. Claim 14 is a dependent claim wherein at least one of the

oligonucleotide primers is labeled. The Examiner acknowledges that "Lindemann et al. do not disclose using bulked segregant analysis to isolate the amplified products and the labeled primer." (Final Rejection dated 3/18/2004, page 4, lines 15-16, emphasis added). The Examiner then cites Halverson et al. as disclosing "a method for sex identification involving bulked segregant analysis (See column 21, lines 23-26) and that the primer used is joined to a label (See column 38, lines 23-25)." (emphasis added). However, Halverson et al. did not involve transposable elements, and more specifically, Halverson et al. does not teach using a primer that selectively hybridizes to a transposable element. Halverson confronts a totally different problem than that confronted by Applicants. The problem confronted by Halverson et al. was to identify nucleotide sequences for sex specific markers that are homologous to genetic sequences on one or both of the Z and W chromosomes of most birds tested. (See Halverson et al. column 2, lines 61-63). In contrast, the problem confronted by Applicants was to efficiently identify a gene of unknown location and sequence whose genomic DNA was disrupted by a transposable element. It is difficult to see exactly what would lead one of ordinary skill in the art to combine Halverson et al. to Lindemann et. al. and Kindiger et al. in order to arrive at Applicant's claimed invention.

The arguments made by the Applicants in connection with the claim rejections under the combination of Lindemann, et al. and Kindiger et al. are equally applicable to the claim rejections under the combination of Lindemann, et al., Kindiger et al. and Halverson et al., and such arguments are hereby incorporated by reference into this section.

The prior art references do not provide a basis for either modifying or combining the references to achieve Applicants' claimed invention. The patent office has not met its burden of establishing how or why a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select and modify the elements from the cited prior art references for combination in the manner claimed. The cited prior art references do not acknowledge the desirability of solving the problems that have been solved by Applicants' invention. Further, even assuming *arguendo* that there is a motivation to combine Lindemann et al., Kindiger et al. and Halverson et al., these three references

together still lack elements of the claimed invention and contain elements that have been eliminated in Applicants' invention.

In view of the foregoing remarks, reversal of the outstanding rejections and allowance of the pending claims is respectfully requested. The Examiner is invited to call the Attorney for Applicants at the number shown below to expedite resolution of this Appeal.

Respectfully submitted,

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APPENDIX OF CLAIMS

The text of the claims in the appeal are:

1. (Previously Amended) A method for the identification and isolation of a genetic sequence from an organism, wherein disruption of genomic DNA of said organism by a transposable element flanking said genetic sequence is associated with a mutant phenotype, said method comprising the following steps:

a) segregating a plurality of organisms by the presence or absence of said mutant phenotype, wherein the genomic DNA of each organism comprises at least one copy of said transposable element;

b) obtaining a mutant genomic DNA sample from at least one of said organisms exhibiting said mutant phenotype and a wild-type genomic DNA sample from at least one of said organisms not exhibiting said mutant phenotype;

c) fragmenting at least one of said mutant and at least one of said wild-type genomic DNA samples to produce DNA fragments;

d) attaching an adapter to at least one of said mutant DNA fragments and to at least one of said wild-type DNA fragments, resulting in a collection of adapter-modified DNA fragments;

e) amplifying said mutant and wild-type adapter-modified DNA fragments to yield amplification products comprising said genetic sequence wherein said amplification employs a first oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said transposable element ; and,

f) isolating an amplification product present in said organism exhibiting said mutant phenotype and absent in said organism not exhibiting said mutant phenotype, wherein said isolated amplification product comprises said genetic sequence associated with said mutant phenotype.

2. (Previously Amended) The method of claim 1, wherein step (f) comprises using cosegregation analysis to isolate said amplification product that cosegregates with said mutant phenotype.

3. (Original) The method of claim 1, wherein step (f) comprises using bulked segregant analysis to isolate said amplification product.

4. (Original) The method of claim 1, wherein said transposable element is a transposable element that comprises a terminal inverted repeat (TIR) sequence.

5. (Original) The method of claim 4, wherein said transposable element is a member of the *Mutator* family of transposable elements.

6. (Previously Amended) The method of claim 5, wherein *Mutator*-TIR is a template for said second oligonucleotide primer .

7. (Original) The method of claim 1, wherein said organisms are plants.

8. (Original) The method of claim 7, wherein said plant is a maize plant.

9. (Previously Amended) The method of claim 1, further comprising a second amplification to preferentially amplify adapter-modified DNA fragments, wherein said second amplification employs at least two oligonucleotide primers, with one of said primers selectively hybridizing under stringent hybridization conditions to

said adapter sequence, and the other primer selectively hybridizing, under stringent hybridization conditions, to said transposable element.

10. (Previously Amended) The method of claim 9, wherein said primers of said second amplification are nested within said primers of step (e) of claim 1.

11. (Previously Amended) The method of claim 1, wherein said amplification of step (e) is achieved by polymerase chain reaction (PCR).

12. (Previously Amended) The method of claim 1, wherein said fragmentation of step (c) is achieved by digestion with at least one restriction enzyme.

13. (Previously Amended) The method of claim 1, wherein said mutant genomic DNA sample of step (b) comprises genomic DNA from at least 2 organisms and said wild-type genomic DNA sample comprises genomic DNA of 10 organisms.

14. (Previously Amended) The method of claim 1, wherein at least one of said oligonucleotide primers is labeled.

15. (Previously Amended) A method for identifying one or more locations of a genomic insertion by a transgene in genomic DNA of an organism, said method comprising the following steps:

a) isolating a genomic DNA sample from said organism;

b) fragmenting said isolated genomic DNA sample to yield a collection of DNA fragments;

c) attaching an adapter sequence to at least one of said DNA fragments to yield a collection of adapter-modified DNA fragments;

d) amplifying said adapter-modified DNA fragments, wherein said amplification employs a first primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second primer which selectively hybridizes under stringent hybridization conditions to said transgene sequence; and

e) analyzing said amplification products to identify the location of said genomic insertion.

16. (Original) The method of claim 15, wherein said organism is a plant.

17. (Previously Amended) The method of claim 15, further comprising a second amplification to preferentially amplify adapter-modified DNA fragments, wherein said second amplification employs at least two oligonucleotide primers, with one of said primers selectively hybridizing under stringent hybridization conditions to said adapter sequence and the other primer selectively hybridizing under stringent hybridization conditions to said transgene.

18. (Original) The method of claim 16, wherein said transgenic organism is a maize plant.

19. (Original) The method of claim 17, wherein said primers are nested with the primers of claim 15.

20. (Original) The method of claim 15, wherein said amplification is achieved by polymerase chain reaction (PCR).

21. (Original) The method of claim 15, wherein the fragmentation of step (b) is achieved by digestion with at least one restriction enzyme.